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Original research article

Naris deformation in Darwin's finches: Experimental and historical evidence for a post-1960s arrival of the parasite *Philornis downsi*

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ABSTRACT

The rate of evolution depends on the strength of selection, which may be particularly strong for introduced parasites and their naive hosts. Because natural selection acts on phenotypes and because parasites can alter host phenotype, one fruitful starting point to measure the impact of novel pathogens is to quantify parasite-induced changes to host phenotype. Our study system is Darwin's finches on Floreana Island, Galápagos Archipelago, and the virulent fly larvae of *Philornis downsi* that were first discovered in Darwin's finch nests in 1997. We use an experimental approach and measure host phenotype in parasitized and parasite-free chicks in Darwin's small ground finch (*Geospiza fuliginosa*). Beak size did not differ between the two treatment groups, but naris size was 106% larger in parasitized chicks (~3.3 mm) versus parasite-free chicks (~1.6 mm). To test if *P. downsi* was present prior to the 1960s, we compared naris size in historical (1899–1962) and contemporary birds (2004–2014) on Floreana Island in small ground finches (*G. fuliginosa*) and medium tree finches (*Camarhynchus pauper*). Contemporary Darwin's finches had significantly larger naris size (including extreme deformation), whereas historical naris size was both smaller and less variable. These findings provide the first longitudinal analysis for the extent of *P. downsi*-induced change to host naris size and show that Darwin's finches, prior to the 1960s, were not malformed. Thus natural selection on altered host phenotype as a consequence of *P. downsi* parasitism appears to be contemporary and novel.

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1. Introduction

Natural selection acts on phenotypes (Endler, 1986; Mayr, 1963), and parasites may alter the phenotype of their hosts (Moore, 2002; Poulin, 2011). For these reasons, parasites can drive population divergence of hosts and increase the rate of evolution among host populations whenever parasites alter host phenotypes (Karvonen and Seehausen, 2012; Maan and Seehausen, 2011; Miura et al., 2006; Schmid-Hempel, 2011). To understand the mutual evolutionary impact of parasites and hosts, it is necessary to measure phenotypic change in hosts and parasites as the outcome of the association. A growing number of examples showcase the remarkable capacity of parasites to change host morphology, host behavior, and host microhabitat selection—presumably to increase parasite fitness (Barber et al., 2000; Bass and Weis, 1999; Combes, 1991; Poulin, 2010; Seppälä et al., 2005; Thomas et al., 2010). But few studies have provided compelling evidence that the

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parasite is the sole cause of the altered host phenotype or measured changes in parasite fitness due to changes in host phenotype (Poulin, 1995). Phylogenetic and detailed species-level insights from host–parasite associations are limited due to the unknown onset of most host–parasite associations in the wild. Clearly, a temporal framework of known host–parasite association would provide a useful benchmark to test evolutionary patterns in hosts and parasites (Blanquart et al., 2013; Kaltz and Shykoff, 1998). Studies with known onset of host–parasite association combined with experimental approaches to identify parasite-induced changes in host phenotype will allow quantification of natural selection on parasite-altered host phenotype (Carius et al., 2001; Clayton et al., 1999; Fessl et al., 2006a).

By definition, parasites consume resources from a host at a fitness cost to the host (Loye and Zuk, 1991). It can be difficult to measure the fitness cost of parasites under conditions of age-specific parasitism, because some effects of parasites may persist in hosts for the short-term while others result in permanent change (Galligan and Kleindorfer, 2009). Parasites can cause short-term deformation in the host, such as tissue damage (which can subsequently heal) and/or long-term malformation caused by parasite-induced developmental instability (Møller, 1996). Hence, depending on parasite-induced deformation or malformation, different host phenotypes are expressed at different developmental stages and for different periods of time (Galligan and Kleindorfer, 2009; Møller, 2006). In order to quantify phenotypic changes in hosts with age-specific parasitism, it is important to distinguish between deformation, which is generally an extrinsic process, and malformation, which is an intrinsic and developmental process, although it may be triggered by extrinsic influences, including initial deformation by a parasite. When malformation has been identified, selection can be measured at different life stages as the malformation persists across an individuals' lifespan after the organism has survived earlier parasitism (Galligan and Kleindorfer, 2009; Goodman and Johnson, 2011; Johnson et al., 2002).

The recently discovered host–parasite association between Darwin's finches and parasitic larvae of *Philornis downsi* on the Galápagos Archipelago provides a timely case study to measure parasite-induced changes in host phenotype on the one hand, and on the other hand, to use this information to assess whether the parasite was present on the Galápagos Islands prior to the 1960s. We focus on this date because the first known *P. downsi* sample is from an insect collection made on Santa Cruz Island in 1964 (Causton et al., 2006; Dudaniec and Kleindorfer, 2006). Given ample historical collections of Darwin's finch species by the Stanford University expedition led by Robert Snodgrass and Edmund Heller (1898–1899), the expedition of the California Academy of Sciences (1899, 1905–1906, 1932), and later by Robert Bowman (in the 1950s and 1960s), one can access historical specimens against which to compare contemporary host phenotype. If historical specimens do not contain *P. downsi*-induced changes in host phenotype, we can conclude that *P. downsi* was introduced post-1960s. Determining the decade of introduction of *P. downsi* is crucial to modeling the strength of selection and rates of evolution in host–parasite associations across Galápagos species and islands in order to predict trajectories for population extinctions and/or local host–parasite adaptedness (Boyer, 2008; Fessl et al., 2010; Jarvi et al., 2001; Kaltz and Shykoff, 1998; Koop et al., 2015).

In 1997, larvae of *P. downsi* were first discovered in Darwin's finch nests on Santa Cruz Island (Fessl et al., 2001), though the adult fly was retrospectively found in insect collections from this same island in 1964 (Causton et al., 2006, 2013). Our study site, Floreana, lies 50 km to the south of Santa Cruz. Both islands have settlements that have long been interconnected by boat travel. Floreana was first settled in 1832, three years before Darwin's historic visit with H.M.S. *Beagle*, and Santa Cruz was colonized in the 1920s, when a group of Norwegians established a fishing cannery on the island (Latorre, 1999). During and after World War II, fishermen and supply vessels were increasingly frequent visitors to these two islands, as well as to settlements located on two other islands. *Philornis* has recently been detected on the Ecuadorian mainland, and it probably arrived from the mainland on a cargo vessel (Bulgarella, 1999). Once introduced – most likely to Santa Cruz Island – this insect would easily have dispersed throughout the archipelago, either by active flight, wind, or conveyance on ships. Two wasp species (*Polistes versicolor* and *Brachygastra lecheguana*) and a species of black fly (*Simulium bipunctatum*) are known to have arrived in the Galápagos in the 1980s and 1990s, and all three species spread from their initial point of introduction to other islands in less than a decade (Heraty and Abedrabbo, 1992; Roque-Albelo and Causton, 1999). *Philornis* is now known to be present on 13 of the 16 major islands in the Galápagos group (Causton et al., 2013).

The *P. downsi* larvae consume the blood and tissue of developing birds and are considered the biggest risk to survival among all Galápagos land birds (Causton et al., 2013; O'Connor et al., 2010d). Among Darwin's finches, in-nest chick mortality due to *P. downsi* has been 3%–100% across years (Cimadom et al., 2014; Dudaniec and Kleindorfer, 2006; Dudaniec et al., 2007; Fessl et al., 2010; Huber, 2008; Kleindorfer et al., 2014b; Knutie et al., 2014; O'Connor et al., 2010d). From previous study, including experimental manipulation and in-nest video cameras, we know that 1st instar larvae reside inside the nares of developing birds where they feed on the soft keratinous tissue (Fessl et al., 2006a,b; O'Connor et al., 2010b, 2014). The 2nd and 3rd instar larvae feed on chicks internally and externally, and they subsequently pupate inside the nest base before emerging as adult flies after 10–14 days (Fessl et al., 2006a,b). The few surviving Darwin's finch chicks fledge with varying levels of naris deformation (enclosed with pupae, normal, or empty and enlarged) that persists into adulthood as varying degrees of naris malformation (Galligan and Kleindorfer, 2009).

In this study of small ground finch (*Geospiza fuliginosa*) and medium tree finch (*Camarhynchus pauper*) on Floreana Island, Galápagos Archipelago, we have two main aims. (1) We use an experimental approach to test the magnitude of naris deformation in developing *G. fuliginosa* chicks due to *P. downsi* parasitism. (2) We also examine naris size among adult *G. fuliginosa* and *C. pauper* across three time periods in order to compare patterns of parasite-induced host malformation across the past century. The historical samples, which are divided into two temporal subsamples, consist of collections made between 1899 and 1962. Contemporary samples are part of an ongoing long-term field study from 2004 to 2014. We test the

prediction that *P. downsi* larval infestation significantly increases chick naris size, and that naris deformation among chicks persists into adulthood (larger average naris size in contemporary birds, including extreme malformation). Additionally, we conduct a variety of statistical tests to determine whether *P. downsi* was present in the Galápagos Archipelago prior to the 1960s, before its first official record of presence in 1964. Although *P. downsi* causes enlarged nares in developing chicks, minimally damaged nares sometimes close over as a result of regrowth following parasite infection—for example, when a parasitic larva remains stuck inside the naris (Kleindorfer unpublished data). We therefore test both for increased naris size over time and for increased naris heterogeneity over time.

2. Material and methods

2.1. Study site

Both contemporary field work and historical specimens are from Floreana Island, Galápagos Archipelago, and were collected from lowland (1°16′S, 90°29′W) and highland (1°17′S, 090°27′W) sites on Floreana Island, as described in our previous work (Dudaniec et al., 2008; O'Connor et al., 2010a). The contemporary data were collected during February (2004, 2005) and during February to April (2006, 2008, 2010, and 2012–2014) (Kleindorfer et al., 2014a,b). The historical specimens were collected in 1899 (during the Stanford University Expedition led by Robert Snodgrass and Edmund Heller), in 1905–1906 and 1932 (during expeditions sponsored by the California Academy of Sciences), and in 1962 (by Robert Bowman).

2.2. Morphological measurements for Darwin's finch species

We collected morphological data in the field from 565 small ground finch (*Geospiza fuliginosa*) and 159 medium tree finch (*Camarhynchus pauper*), listed as critically endangered by the IUCN. The medium tree finch is endemic to Floreana Island and only occurs in the small remnant patch of highland *Scalesia* forest (Kleindorfer et al., 2014a; O'Connor et al., 2010c; Peters and Kleindorfer, 2015). The small ground finch is perhaps the most common Darwin's finch species and is amply abundant and widely distributed across habitats and islands (Galligan et al., 2012; Galligan and Kleindorfer, 2010; Kleindorfer et al., 2006; Sulloway and Kleindorfer, 2013).

To measure morphology and naris size in the field, we captured finches using mist-nets. Birds were subsequently banded with a numbered aluminum ring and a unique combination of color bands. Each year we placed six 12 m mist-nets between 05:30–1100 h along the 2 km mist-netting transect, sampled the location once, and moved all nets ∼100 m the next day. We measured the following morphological traits (mm) for all birds mist-netted: (1) beak length from the anterior edge of the naris opening, (2) beak depth at base, (3) beak width at base, (4) wing length (the flattened wing), and (5) size of the right naris (measured at the widest point). In colloquial terms, the "naris" is the nasal opening of the beak.

Museum specimens of *C. pauper* ($n = 195$) and *G. fuliginosa* ($n = 88$) were measured at the California Academy of Sciences, which houses the world's largest collection of Darwin's finches (more than 5000 specimens). Although the bulk of the historical specimens from Floreana ($n = 250$) were collected during the Academy's expedition in 1905 and 1906, our analysis also includes 3 specimens of *C. pauper* collected in 1899 during the Hopkins-Stanford expedition, 1 specimen of *G. fuliginosa* collected in 1932 during the Academy sponsored Templeton Crocker expedition, and 29 specimens of *C. pauper* collected by Robert Bowman in 1962. The second author measured the same morphological traits that were measured for field specimens, using the same measurement protocols. For these historical specimens, as many as six measurements were taken of each naris and then averaged, yielding a reliability of 0.80 (Cronbach's α). For a subset of specimens (both those collected in the field and those measured at the California Academy), we also measured the left naris in order to assess possible asymmetry. For the historical and modern specimens as a whole, the correlation between the two nares was 0.70 for *G. fuliginosa* and 0.53 for *C. pauper*. Given the increased reliability inherent in using measurements of both nares, we combined these measures in all statistical analyses whenever both were available. For all specimens included in the study, these composite measures of naris size yielded a Cronbach's α of 0.77 for *G. fuliginosa* and 0.64 for *C. pauper*.

Because measurements made by three field assistants entailed a slightly different measurement protocol (whether to include the beginning of indentation in the culmen, just before the full extent of the naris opening is manifested), these measurements differed systematically from those made by the first author (being 0.40 mm smaller, on average). This average difference was consistently present in four different species of Darwin's finches (*G. fuliginosa*, *G. fortis*, *C. parvulus*, and *C. pauper*) as well as among 23 recaptured specimens measured by the first author as well as by the three field assistants ($t = 6.20$, $df = 42.8$, $p < 0.0001$). For this reason we applied a correction factor to the measurements made by the field assistants in order to make measurements consistent among all of the modern specimens. This adjustment consisted of multiplying the naris measurements made by the three field workers by a factor of 1.27 for *C. pauper* (corresponding to an average difference of 0.40 mm), and by a factor of 1.30 for *G. fuliginosa* (corresponding to an average difference of 0.39 mm). As a sensitivity check on the validity of these adjustments, we conducted statistical analyses that excluded all 240 birds measured solely by field assistants. Because our principal finding and conclusions are confirmed by analyses restricted to this smaller subsample ($n = 400$), we report here only the findings derived from the entire sample.

2.3. Parasite species

Philornis downsi is a parasitic Dipteran whose larvae have two temporally distinct feeding modes: first instar larvae feed internally on the nasal cavities inside the nares of its avian nestling hosts, and 2nd and 3rd instar larvae mostly feed externally on the chicks (Fessl et al., 2006a,b; O'Connor et al., 2010b, 2014). After nesting outcome is known in Darwin's finches, we collect the nest and examine the contents for *P. downsi* larvae and pupae and identify the instars based on their size and distinguishing features (Fessl et al., 2006a,b; Kleindorfer et al., 2014b).

The genus *Philornis* has a Neotropical distribution comprised of ∼50 species (Bulgarella et al., 2015; Dudaniec and Kleindorfer, 2006; Quiroga et al., 2012). How *P. downsi* arrived in the Galápagos Islands is not known, but two likely scenarios have been suggested: (1) introduction via known mainland hosts such as Smooth-billed Ani (*Crotophaga ani*) that was introduced to the Galápagos Islands in 1962 (Rosenberg et al., 1990; Santiago-Alarcon et al., 2006; Thiel et al., 2005; Wikelski et al., 2004); and (2) given that adult *P. downsi* feeds on fruit, the fly may have arrived via cargo boats carrying produce from the mainland Ecuador (Causton et al., 2013; Dudaniec et al., 2007). Recently, *P. downsi* was discovered in bird nests on mainland Ecuador (Bulgarella et al., 2015), which renders the possibility that it was accidentally introduced by cargo boats more plausible.

2.4. Experimental effects of P. downsi on naris size

In 14 nests of small ground finch (*G. fuliginosa*) in 2010, we applied pyrethrum spray to 8 experimental nests and water spray to 6 control nests to create "parasite-free" and "parasitized" nests respectively. Both clutch size (3–4 eggs) and number of two-day old chicks were comparable across treatment groups. For the experimentally parasite-free nests, we removed 2-day-old chicks, sprayed the nest interior with 1% pyrethrum solution, and returned the chicks after 10 min. Pyrethrum appears to be nontoxic to Galápagos birds and virtually eliminates larvae that are already present in the nest (Causton and Lincango, 2014; Fessl et al., 2006a,b; O'Connor et al., 2014). After spraying with pyrethrum, *P. downsi* flies have not been observed to re-enter nests, which we confirmed using continuous in-nest video recordings (O'Connor et al., 2010b). For the parasitized group, we removed 2-day old chicks, sprayed the nest interior with water, and returned the chicks after 10 min. We measured naris size every two days in developing chicks by placing calipers at the two outermost sides of the naris opening for left and right naris; for the analysis we used the mean naris size for left and right naris (mm) per chick. The data analyzed in this study are largely the same as those reported in O'Connor et al. (2014), who found differences in composite beak and body size PCA factor scores as well as naris size in chicks from 7 parasitized and 7 parasite-free nests. In this study we focus on the effect of *P. downsi* for beak phenotype (Galligan and Kleindorfer, 2009) and analyze beak length from naris to tip (mm) and mean naris size (mm) (using the average naris size for left and right naris per chick), as well as relative naris size in relation to beak length.

2.5. Statistical analysis

Data were analyzed with SPSS 22 for Windows (SPSS Inc., Chicago, USA), Stata 14 for Windows (College Station, Texas, USA), and SYSTAT 13 for Windows (San Jose, California, USA). Before conducting statistical analyses, we examined the data to determine if they conformed to assumptions of normality and homogeneity of variance. Morphology data were log transformed to satisfy requirements of normality. Because significant heterogeneity remained in naris measurements for both species, after log transformations, we performed all regression analyses in Stata 14 using robust standard errors, which relaxes the assumption that errors are independent and identically distributed, producing more reliable estimates and significance tests. In addition, planned contrasts were adjusted for multiple testing, using Scheffe's correction when Levene's test for the equality of variances was not rejected, and Dunnett's T3 test when Levene's test was rejected. The experimental data for parasite effects within nests were analyzed using multilevel modeling, to control for the clustering of data by nest.

3. Results

3.1. Experimental effects of P. downsi on naris size

Beak length did not differ significantly across treatment groups (parasite-free, parasitized) for any age class during chick development (Table 1). Naris size differed significantly across treatment groups (Table 1). Chicks from parasite-free nests (treated with insecticide) had significantly smaller naris size after day 3–4 compared with parasitized chicks whose nests had been sprayed with water (Table 1). As beak length did not differ across treatment groups, we found the same pattern of change in relative naris size as we did for absolute naris size (Table 1).

3.2. Differences in naris size: contemporary versus historical specimens

We used linear regression to analyze changes in naris size over time. For both species of Darwin's finches, there was a significant increase in naris size as well as heterogeneity, from 1899 to 2014 (Fig. 1). For *C. pauper*, the nares of modern

Table 1
The effects of *Philornis downsi* larvae for (a) beak length (mm) and naris size (mm) in developing chicks at experimental nests with and without *P. downsi* larvae. Relative naris size (%) was calculated in relation to beak length (naris size/beak length × 100) and is shown in brackets below absolute naris size (mm). Beak length did not differ significantly between treatment groups, but parasite-free chicks (pyrethrum treatment; $n = 8$ nests) had smaller naris size than parasitized chicks (water treatment, $n = 6$ nests) after 3–4 days. Results of multilevel modeling are presented, along with sample sizes for number of nests and number of chicks per age class.

(a) Beak length (mm) in parasite-free versus parasitized nests.

Chick age (days)	Beak length (mm)		*t*-Tests for naris size (mm)	
	Parasite-free (0 *Philornis per nest*)	Parasitized (24.2 ± 2.7 *Philornis* per nest)	*t*-value	*P*-value
1–2 days	4.63 ± 0.13 (7 nests, 22 chicks)	4.54 ± 0.14 (3 nests, 13 chicks)	0.34	0.75
3–4 days	5.65 ± 0.12 (8 nests, 28 chicks)	6.00 ± 0.26 (4 nests, 13 chicks)	−0.33	0.75
5–6 days	6.51 ± 0.15 (7 nests, 23 chicks)	5.80 ± 0.37 (2 nests, 4 chicks)	1.63	0.14
7–8 days	7.45 ± 0.30 (4 nests, 12 chicks)	7.65 ± 0.25 (2 nests, 2 chicks)	−0.58	0.96
9–10 days	8.00 ± 0.23 (4 nests, 16 chicks)	8.40 ± 0.33 (2 nests, 4 chicks)	−0.21	0.84

(b) Naris size (mm, % relative to beak length) in parasite-free versus parasitized nests.

Chick age (days)	Naris size (mm) (% Naris size relative to beak length)		Independent *t*-test for naris size (mm)	
	Parasite-free (0 *Philornis*)	Parasitized (24.2 ± 2.7 *Philornis* per nest)	*t*-value	*P*-value.
1–2 days	1.34 ± 0.15 (28.1 ± 3.0)	1.58 ± 0.13 (34.5 ± 2.5)	−0.94	0.37
3–4 days	1.81 ± 0.09 (32.2 ± 1.6)	2.29 ± 0.16 (38.0 ± 2.1)	−2.98	0.12
5–6 days	1.81 ± 0.10 (28.3 ± 2.1)	2.36 ± 0.26 (41.7 ± 6.7)	−2.53	0.018
7–8 days	1.88 ± 0.17 (25.6 ± 2.7)	2.68 ± 0.28 (35.1 ± 4.7)	−1.61	0.13
9–10 days	1.62 ± 0.18 (20.2 ± 2.2)	3.28 ± 0.17 (39.0 ± 1.3)	−3.93	0.007

specimens were larger than those observed among the historical specimens (Fig. 2, 1.75 mm versus 1.91 mm, $SD = 0.18$; $r = 0.29, t = 4.96, n = 302, p < 0.0001$). Based on a Breusch–Pagan/Cook–Weisberg test for heteroskedasticity, variances in *C. pauper* were found to increase linearly with year of collection ($\chi^2_1 = 51.91, r_{pb} = 0.41, n = 302, p < 0.0001$). For this same finch species we also performed a planned contrast comparing the 29 specimens collected by Robert Bowman in 1962 with those captured between 2005 and 2014. Although naris size among Bowman's specimens did not differ from those birds collected between 1899 and 1906 ($r_{pb} = 0.00, t = 0.02, n = 186, p = 0.98$), they did differ from the birds captured and measured after 1962 ($r_{pb} = 0.25, t = 3.11, n = 145, p = 0.002$). Similarly, naris-size heterogeneity was significantly greater among modern specimens than among those collected by Bowman in 1962 (Breusch–Pagan/Cook–Weisberg test, $\chi^2_1 = 9.57, r_{pb} = 0.26, n = 145, p = 0.002$). Consistent with this last finding, Bowman's specimens did not differ in heterogeneity from other historical specimens (Breusch–Pagan/Cook–Weisberg test, $\chi^2_1 = 0.43, r_{pb} = 0.05, n = 186, p = 0.51$).

In our analyses of *G. fuliginosa*, we also found a linear trend for increasing naris size (Fig. 1(b)). The nares in modern specimens were 0.63 SD larger than those in historical specimens (Fig. 2: 1.57 mm versus 1.76 mm, $SD = 0.30$; $r_{pb} = 0.24, t = 5.61, n = 526, p < 0.0001$). A Breusch–Pagan/Cook–Weisberg test again exhibited a linear increase in heteroskedasticity by year of collection ($\chi^2_1 = 23.49, r_{pb} = 0.21, n = 526, p < 0.0001$). Because no specimens of *G. fuliginosa* were collected on Floreana between 1932 and 1962, with only a single specimen procured in 1932, we were unable to test for post-1906 changes in naris size. We did, however, test for linear trends in size and heterogeneity among all of the historical specimens of *G. fuliginosa* (1905–1906), and we found no significant differences (all $ps > 0.50$).

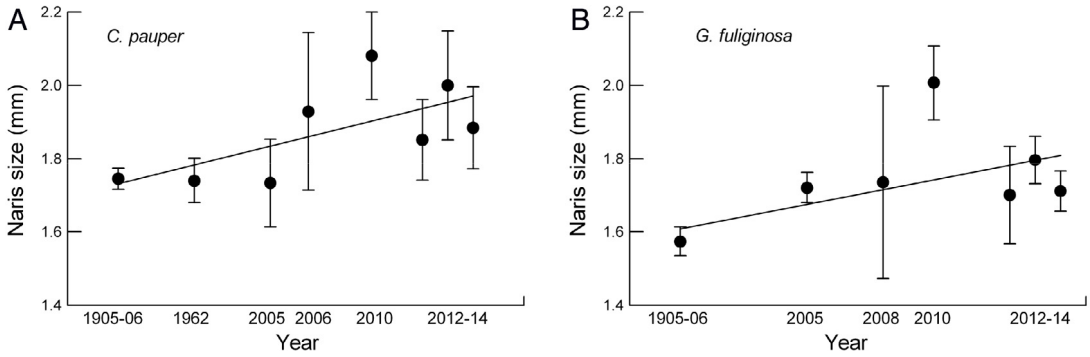

Fig. 1. Significant increases in naris size across years in Darwin's finches on Floreana Island: (a) *C. pauper* ($n = 302$); (b) *G. fuliginosa* ($n = 526$). Error bars indicate 95% CIs (2 × SE). Significant increases in heteroskedasticity over time are evidenced by the relative size of error bars.

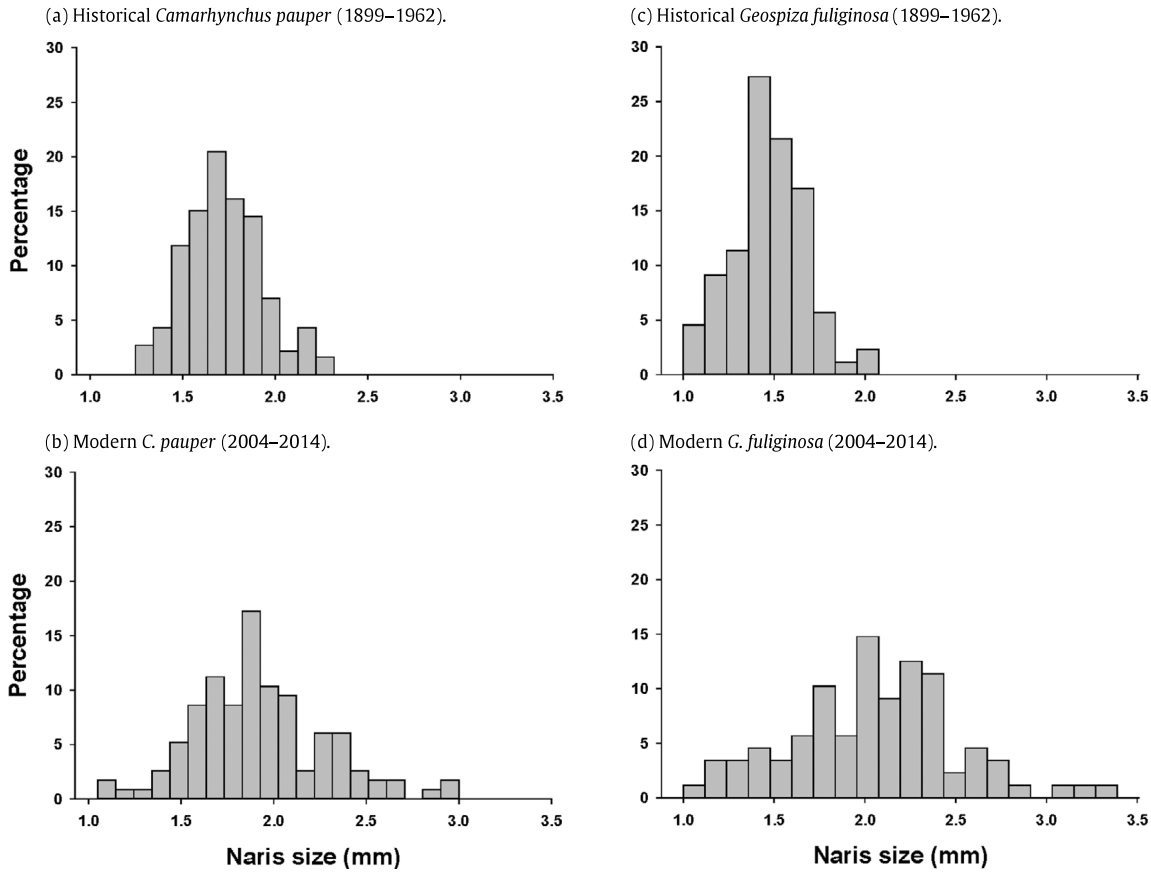

Fig. 2. Histograms showing the percentage distribution of naris size (mm) in medium tree finch (*Camarhynchus pauper*) for (a) 186 historical specimens collected during 1899–1962, and (b) 116 modern birds measured during 2004–2014 on Floreana Island, Galápagos Archipelago; (c) histograms showing the percentage distribution of naris size (mm) in small ground finch (*Geospiza fuliginosa*) for 88 historical specimens collected during 1899–1962, and for (d) 438 modern birds measured during 2004–2014 on Floreana Island, Galápagos Archipelago.

3.3. Proportion of birds with extreme naris size

After binning the naris-size data into 10 subgroups, we examined the 2 most extreme subgroups. Consistent with our findings of greater heteroskedasticity among modern specimens, there was a significant difference in the proportion of birds exhibiting extreme naris size across historical and contemporary specimens. Compared with historical specimens, modern birds were 1.9 times more likely to manifest extreme naris size among birds in the two species combined (23.8% versus 12.4%–$\chi^2_1 = 14.91$, $n = 828$, $phi = 0.13$, $p = 0.0001$; Table 2 and Figs. 1–2). Most of this difference is attributable to birds having enlarged nares, with modern specimens being a striking 19.8 times more likely to fall into this category (χ^2_1 with

Table 2
A comparison of extreme naris size in historical versus modern Darwin's finch specimens.

Species	Small naris (≤10%)	Normal naris (80%)	Enlarged naris (≥90%)	Extreme nares (small plus large) (20%)	*N* (100%)
Small ground finch					
Historical	13 (14.8%)	75 (85.2%)	0 (0%)	13 (14.8%)	88 (100%)
Modern	39 (8.9%)	343 (78.3%)	56 (12.8%)	95 (21.7%)	438 (100%)
Medium tree finch					
Historical	19 (10.2%)	165 (88.7%)	2 (1.1%)	21 (11.3%)	186 (100%)
Modern	13 (11.2%)	79 (68.1%)	24 (20.7%)	37 (31.9%)	116 (100%)
Both species (historical)	32 (11.7%)	240 (87.6%)	2 (0.7%)	34 (12.4%)	274 (100%)
Both species (modern)	52 (9.4%)	422 (76.2%)	80 (14.4%)	132 (23.8%)	554 (100%)

Note. Birds were binned by naris size into 10 subgroups to facilitate a comparison between naris size among birds in the two most extreme subgroups: Small nares were ≤1.24 mm for *G. fuliginosa*, and ≤1.33 mm for *C. pauper*; enlarged nares were ≥2.20 mm for *G. fuliginosa*, and ≥2.40 mm for *C. pauper*.

Yate's correction $= 37.10$, $n = 828$, $phi = 0.21$, $p < 0.0001$). Given that our data included measurements for both the left and right nares, we also conducted tests to determine if the degree of asymmetry in naris size was greater in the modern than in the historical specimens—which we anticipated because *Philornis* larvae are not always distributed evenly within the two nares. This hypothesis was confirmed for both species (*G. fuliginosa*, $r_{pb} = 0.11$, $t = 2.30$, $n = 313$, $p = 0.02$; and *C. pauper*, $r_{pb} = 0.13$, $t = 2.08$, $n = 261$, $p = 0.04$). In addition, we found a significant increase in naris asymmetry between the *C. pauper* specimens collected by Robert Bowman in 1962 and those collected after 2000 ($r_{pb} = 0.26$, $t = 3.50$, $n = 105$, $p = 0.001$).

4. Discussion

This study supports the conclusion that the fly *P. downsi* was most likely introduced to Floreana Island, and the Galápagos Islands in general, sometime between 1962 and 2000. Compared with museum specimens belonging to two different species of Darwin's finches on this same island (1899–1962), modern birds have a substantially higher proportion of severely enlarged nares, as well as substantially greater heterogeneity and asymmetry in naris size—all telltale indications of *Philornis*'s presence. In the case of the medium tree finch, there were substantial increases in the same three measures between birds collected in 1962 and those measured after 2000. By contrast, no significant changes in either of these three measures were found across any of the historical samples collected between 1905 and 1962. These findings about the effects of *Philornis* on naris size are complemented by experimental evidence for parasite-induced changes to host phenotype. More specifically, compared with unparasitized chicks of Darwin's finches, we found that chicks infested with *P. downsi* exhibit 106% larger naris size (no other body-size variable differed between treatment groups).

The first record of *Philornis* in 1964 does not rule out an earlier introduction sometime between the early 1960s and the previous collecting efforts of the California Academy of Sciences expedition (1905–1906) as well as of other scientific expeditions during the next five decades. Although our study is limited to a single island and cannot provide a definitive date for the arrival of *Philornis* in the Galápagos, the information presented here represents an important supplement to evidence supplied by the first recorded collections of *Philornis* in 1964. The magnitude of the documented naris deformation in birds after 1962, and its clear absence in birds collected on Floreana in 1962, furnish a useful benchmark against which we may assess the spread of *P. downsi* following its unknown date of introduction. More specifically, Bowman's 29 specimens of *C. pauper* collected on Floreana in 1962 provide 0.92 power to detect an effect that is only half the magnitude of the actual difference in naris-size heterogeneity that we have reported here; and the same 29 birds provide more than 0.80 power to detect a difference just one-third this magnitude. Considerations of statistical power therefore support the conclusion that, even if *Philornis* was present in the Galápagos prior to the early 1960s, the fly had not yet built up sufficient numbers to cause even a small rate of deformation in finch naris size. Given this evidence, together with the known rapidity with which other introduced insects have dispersed within the Galápagos (Roque-Albelo and Causton, 1999) and also the genetic similarity among the different island populations of *Philornis* (Dudaniec et al., 2008), it seems probable that the fly first colonized Floreana sometime between the mid-1960s and 2000, after having spread from Santa Cruz, 50 km to the north. This temporal scenario is consistent with the fact that 84% of the first recorded dates for introduced insects in the Galápagos have occurred after 1960, and these new records directly parallel the accelerating growth of the human population over the next few decades, and especially with the advent of organized tourism beginning in 1970 (Causton et al., 2006; Peck et al., 1998). In addition, a total of 16 collecting expeditions took place after 1906 and before 1960 (6 expeditions during the 1920s, 9 during the 1930s, and 1 in the 1950s); and none of these 16 expeditions recorded the presence of *Philornis* (Linsley and Usinger, 1966). The first direct physical evidence for *P. downsi* on Floreana Island comes in 2004 and 2005 from systematic sampling by Wiedenfeld et al. (2007), by which time the deformation effects of *Philornis* on nares had become unmistakable among the various finch populations inhabiting this and other islands within the archipelago.

There are two explanations for the observed changes in naris heterogeneity that are associated with *P. downsi*: (1) extremely small naris size is caused by *P. downsi* larvae remaining lodged inside the naris (Kleindorfer unpublished data), and (2) extremely large naris size is caused by 1st instar *P. downsi* consuming the soft keratinous beak tissue. Relevant to the first of these two explanations, we observed Darwin's finch chicks and adult birds with completely enclosed and swollen

nares (Fessl et al., 2006a,b; Galligan and Kleindorfer, 2009); among the swollen nares of some chicks, we could identify encrusted larvae that had not emerged (Kleindorfer unpublished data). Therefore, our observations of both extremely small and large naris size in contemporary birds are consistent with *P. downsi* larvae residing inside the nares (resulting in larger naris size), but sometimes failing to successfully emerge to pupate (and hence resulting in small naris size).

The first instar larvae of *P. downsi* reside inside the nares of developing Darwin's finch chicks, from where they consume the blood and soft keratinous beak tissue of developing host chicks (Fessl et al., 2006b). In-field examination of developing chicks informed this conclusion, which was subsequently substantiated with in-nest video (O'Connor et al., 2010b, 2014). Therefore, our finding that naris size was significantly enlarged in chicks being consumed by *P. downsi* is consistent with our understanding of *P. downsi* biology. There is ample evidence for detrimental effects of *P. downsi.* These detrimental effects include a 56% reduction in chick body mass (Fessl et al., 2006a), 18%–55% overnight chick blood loss (Fessl et al., 2006b), 28% lower hemoglobin concentration (Fessl et al., 2006a), 0.80 decrease in chick hemoglobin concentration per additional *P. downsi* larva (Dudaniec et al., 2006), 30% or greater reduction in primary feather length (Koop et al., 2011), and 3%–100% in-nest chick mortality (Cimadom et al., 2014; Fessl et al., 2006a,b; Huber, 2008; Kleindorfer et al., 2014b; Knutie et al., 2014; Koop et al., 2011; O'Connor et al., 2010d). Damage to the beak can have a variety of harmful effects, as beaks are used for feeding, preening, and song; and birds with deformed beaks may have altered diets (van Hemert et al., 2012) and, in the case of Darwin's finches, altered song.

In absolute terms, naris size was 106% larger in parasitized chicks than parasite-free chicks. Parasitized chicks also had significantly larger relative naris size (39%), which we calculated as average naris size (3.3 mm) in relation to average beak length (see Table 1). In parasite-free chicks, average naris size was smaller (1.6 mm) and relative naris size was 20% (Table 1). While relative naris size differed significantly across the two groups, beak length did not.

Having an accurate timeline for the introduction of *Philornis* is of potential importance in understanding certain aspects of coevolution between parasite and host. The "parasite manipulation hypothesis" predicts that a parasite enhances its own transmission by altering host behavior or phenotype (Moore, 2002; Poulin and Thomas, 1999; Thomas et al., 2005). Poulin (2010) questioned the evidence for adaptive host–parasite associations: there must be evidence of "purposive design" and the association must have arisen independently in several lineages. The chick deformation and adult malformation we observed in Darwin's finches from *P. downsi* does not obviously contribute to increased probability of parasite transmission. However, it is possible that *P. downsi* harbors other parasites that could benefit from early host death or larval feeding sites inside host birds (Poulin, 2010).

5. Conclusions

Given the iconic status of Darwin's finches in the development of evolutionary theory, and given the devastating effects associated with the ongoing impact *P. downsi* on Darwin's finches and other Galápagos land birds, increased understanding of the biological consequences of this introduced ectoparasite has become a top conservation research priority (Kleindorfer and Dudaniec, in press). The strength of this study lies in its combination of experimental cross-sectional data and historical longitudinal data, which demonstrate that *P. downsi* causes beak deformation, and that this introduced agent of selection likely did not occur on Floreana Island before the 1960s. This case-study provides a known onset against which to measure evolutionary trajectories in a novel and rapidly evolving host–parasite association.

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